SAR Journal of Anatomy and Physiology

Abbreviated Key Title: *SAR J Anat Physiol*
Home page: https://sarpublication.com/journal/sarjap/home
DOI: https://doi.org/10.36346/sarjap.2024.v05i04.002

ISSN 2707-7713 (P)
ISSN 2709-6874 (O)

Original Research Article

Investigation of the Anatomy of the Arteria Testicularis Supplying the Testis in Cattle and Ram by Plastic Injection and Corrosion Method

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Article History: | Received: 22.10.2024 | Accepted: 27.11.2024 | Published: 29.11.2024 |

Abstract: ***Introduction***: In our study, we aimed to investigate anatomy of testicular artery in cattle and ram by plastic injection and corrosion cast method. ***Materials and Methods:*** In our study, cattle and rams were used. The testes were cleaned, paying attention to the vascular and parenchymal structure. Then testicular artery was cannulated and prepared for plastic injection. Polyester was injected into the testiculary artery. The testes were kept at room temperature for 48-72 hours polyester in the vessel. The obtained corrosion casts were examined macroscopically. ***Results and Conclusions:*** When the course of arteria testicularis is examined macroscopically; on the surface of tunica albuginea arteria testicularis was found to divide 80%, in cattle and 60% in the rams. In general, in the corrosion casts of both species, the arterial testicularis showed very convoluted distribution in the funiculus spermaticus. In both species, when the arteria testicularis reaches the extremitas inferior of the arterial testis, two main branches are separated. These branches were observed to form a vascular network on the surface of tunica albuginea. As a result; although the course of the testicular artery in both species is similar, there are differences.

Keywords: Testis, artery, plastic enjection, cattle, ram.

INTRODUCTION

In mammals, the testes are the most important organ responsible for reproduction after sexual maturity. They are located in pairs in a sac called the scrotum. Arterial blood supply to the testes is closely related to reproduction. Arterial blood supply to the testes is provided by the a. testicularis.

The a. testicularis originates from the aorta abdominalis and is adjacent to important structures along its course (Tunalı and Başar, 2012). Passing through the inguinal canal and reaching the scrotum, the a. testicularis branches on the testicular surface and towards the inner part of the testis and distributes in the mediastinum testis. The a. testicularis penetrates the tunica albuginea along the posterior surface of the testis to form the a. capsularis. The a. capsularis penetrates deeper into the tunica albuginea to form the a. centripetalis. These arteries form a network in the mediastinum of the testis and distribute radially from the center to the periphery. The branches formed in this way are called a. centrifugalis (Lin, *et al.*, 2007; Gutzschebauch 1936; Hofman, 1960; Hundeiker, 1960; Takyama, 1984; Waites and Moule, 1960). Knowledge of the anatomical course of these branches is important in terms of preventing damage to vascular structures during interventional procedures. However, due to the variations seen in the a. testicularis with a rate of approximately 4.7%, attention should be paid to these variations during interventional procedures (Tunalı and Başar, 2012).

In recent years, radiographic methods have been widely used in the examination of the vessels. However, radiographic methods do not provide a complete determination of the structural features, anastomoses and variations of the vessels. The corrosion method has been used in vascular research for centuries (Hassa, 1967). Therefore, examination of vessels by injection of plastic material has gained more importance. In many studies, plastic materials have been used for the extraction of vascular three-dimensional models of normal organs and tissues, determination of pathologic cases, examination of developing structures, cadaver dissection and anatomy education. In our study, polyester was used because it is abundantly available in the industry, cheap and easily accessible.

In our study, we aimed to examine the three-dimensional anatomy and course of the a. testicularis, which supplies the testicles of cattle and rams.

MATERIALS AND METHODS

In our study, 10 cattle and 10 ram testes obtained from slaughterhouses in and around Kayseri were used. Animals aged 24-48 months were selected for plastic injection into the testes. Before the injection procedure, the vascular and parenchymal structure of the testes were dissected and the a. testicularis was exposed. The a. testicularis was then cannulated and prepared for plastic injection. Polyester solution was prepared by adding 70% Polyester resin (Turquoise Corolla Styrene Monomer TPY001), 30% Liquefier (styrene), 2% Accelerator, 4% Freezer, Dye (Red dye). The prepared polyester solution was placed in a 50 ml syringe and injected until all branches of the cannulated a. testicularis were exposed. During injection, the a. testicularis was tied with a string to prevent the solution from escaping between the cannula and the a. testicularis. The testicles were kept at room temperature for 48-72 hours for the polyester to harden in the vessel. After solidification, the testes were placed in 37.5% hydrochloric acid (HCL).

The testes were kept in hydrochloric acid for 48-72 hours. Thus, the tissues were allowed to dissolve. The obtained materials were washed in tap water to remove tissue residues. As a result, molds of a. testicularis were obtained. Macroscopically and microscopically, the patterns of the obtained a. testicularis were analyzed (Aycan, 1990).

RESULTS

In our study, 10 cattle and 10 ram testes were examined. Polyester was injected into the a. testicularis of each testicle. Corrosion vascular casts were removed. It was determined that the a. testicularis was completely filled with plastic material in 8 cattle and 6 ram testes included in the study.

1- Anatomical Course of a. testicularis in the Funiculus Spermaticus

In the corrosion casts of both species, the a. testicularis showed a very convoluted distribution within the funiculus spermaticus. With the overlapping of these folds, it had the appearance of a cone with the top up and the bottom down. It was determined that this cone-shaped arterial bundle was composed of sharp and different geometrical folds. It was also observed that this arterial bundle expanded from the proximal to the upper part of the testicle (Figure 1).

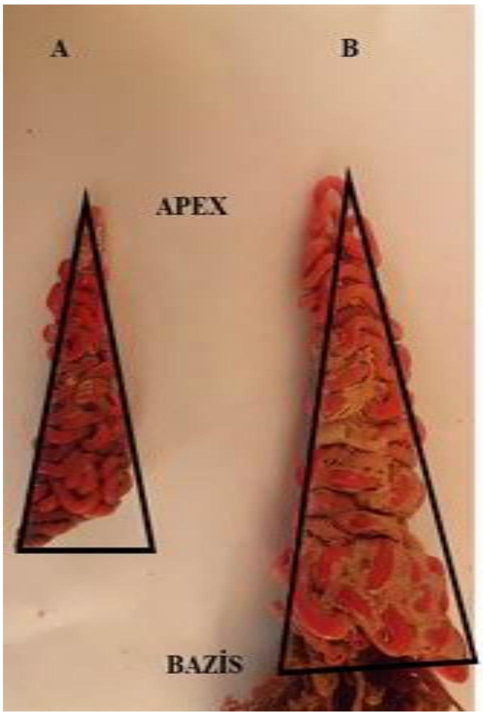

Figure 1: Image of the tortuous course of the a. testicularis in the funiculus spermaticus
(A- corrosion cast of ram testis, B- corrosion cast of cattle testis)

The a. testicularis funiculus spermaticus of both species showed three different types of folds along its course. These were; a- intertwined folds without a specific shape (irregular), b- spiral folds in the shape of the letter "S" c- straight course folds (Figure 2). They then left the funiculus spermaticus and traveled towards the testis.

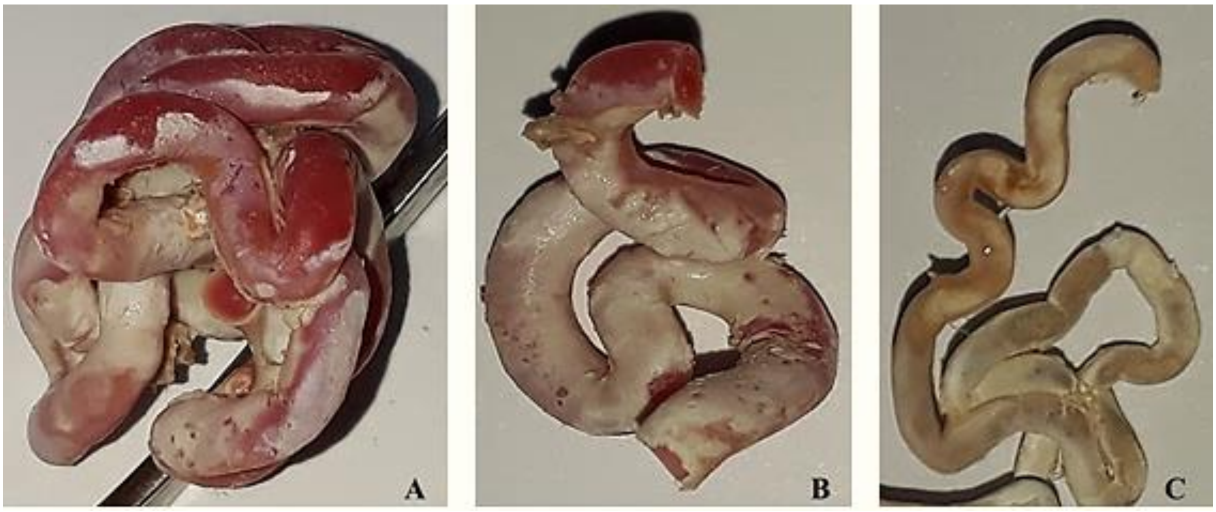

Figure 1: Types of folds in the funiculus spermaticus of a. testicularis (A- Irregular fold, B- Spiral fold, C-Straight fold)

The cone-shaped folds along the funiculus spermaticus were observed to sit like a hat on the extramitas superior of the testis. The arrangement of these folds between species was found to be convex and similar to a daisy flower pattern (Figure 3).

Figure 3: View of the cone-shaped a. testicularis from the base of the cone (A- Corrosion cast of cattle testis, B- Corrosion cast of ram testis)

2-Course of a. testicularis along the Margo Posterior of the Testis

In both species, as the a. testicularis in the funiculus spermaticus left the cone-shaped vascular bundle structure, its tortuosity along the margo posterior decreased. However, some of its branches also showed reduced convolutions (Figure 4).

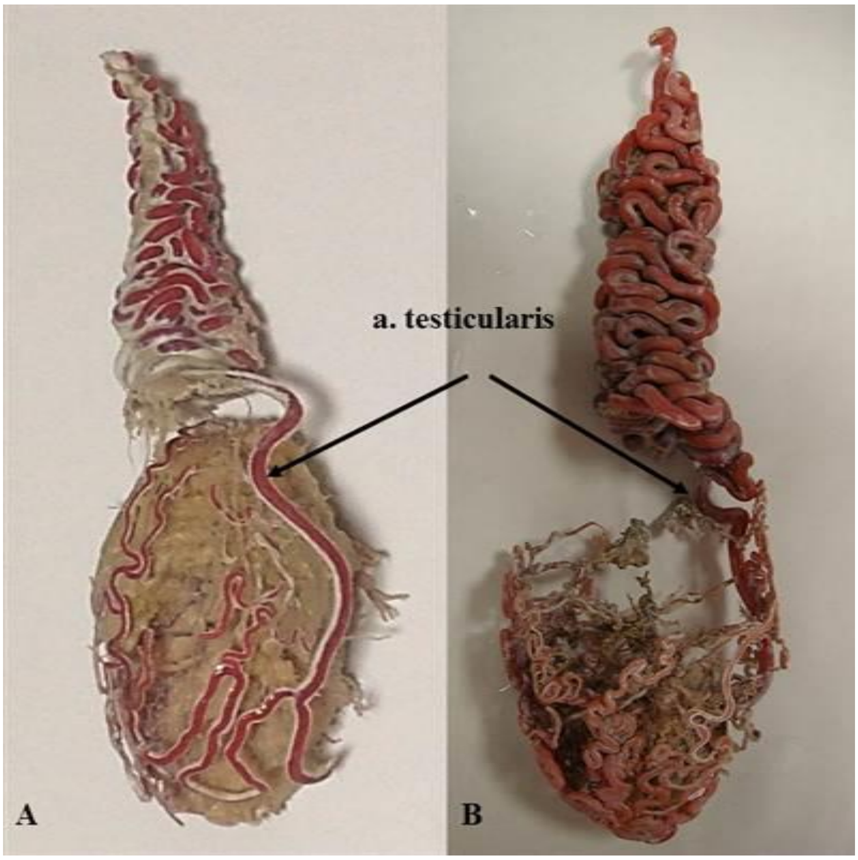

Figure 4: The course of a. testicularis along the margo posterior of the testicle (A- Corrosion cast of the ram testicle, B- Corrosion cast of the cattle testicle)

Although the course of a.testicularis along the margo posterior of the testicle was similar in both species, it was determined that it coursed in a straight, zigzag, curved manner or with structures that did not have a specific shape (Figure 5).

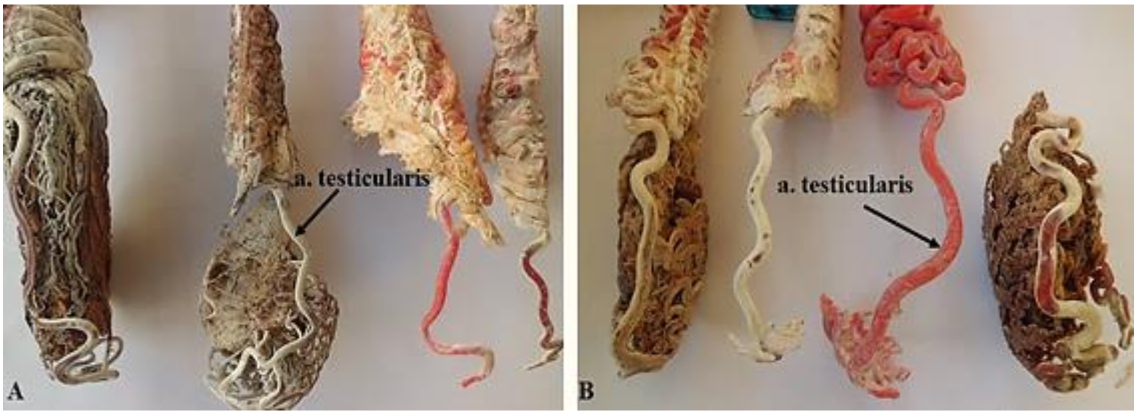

Figure 5: Different types of course of A.testicularis along the margo posterior of the testis (A- corrosion cast of ram testis, B- corrosion cast of cattle testis)

3-A. Distribution of Testicularis on the Surface of Tunica Albuginea

In both species a. It was observed that testicularis divided into two main branches when it reached the extremitas inferior of the testis. It was determined that these branches formed an oval vascular network on the superficial side of the tunica albuginea. It was determined that this vascular network took the shape of the testicle and surrounded the testicle in an oval shape of variable density and diameter, from the extremitas inferior to the extremitas superior and from the facies medialis to the facies lateralis (Figure 6, 7).

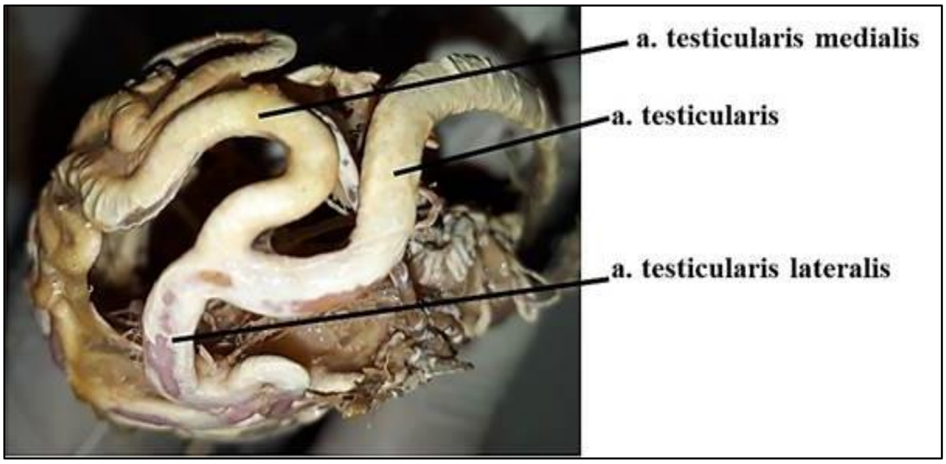

Figure 6: View of a. testicularis from the extremitas inferior of the testis

In both species, the course of the a. testicularis was macroscopically found to divide into two similar terminal branches superficial to the tunica albuginea (Figure 7). The branch extending towards the facies lateralis of the testis was found to be a. testicularis lateralis and the branch extending towards the facies medialis was found to be a. testicularis medialis. However, no anastomosis or vascular variant was observed between these branches surrounding the testis in both species (Figure 8).

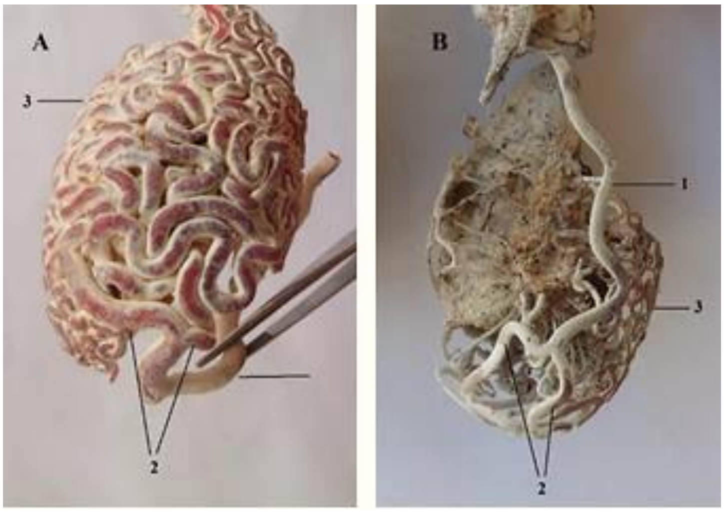

Figure 7: Distribution of the a. testicularis on the surface of the tunica albuginea (A- corrosion cast of the cattle testis, B- corrosion cast of the ram testis, 1- a. testicularis, 2- division of the a. testicularis into two branches (a. testicularis lateralis and medialis) 3- a. testicularis lateralis)

Figure 8: Picture showing the absence of anastomosis between the branches of the a. testicularis (A- corrosion cast of cattle testis, B- corrosion cast of ram testis)

4- Epididiydymal branches of a. testicularis

In our study, the branches supplying the epididymis of both species were examined (Figure 9). In both species, the vessel numbered 1 is the a. epididymalis anterior, which branches from the a. epididymalis and runs along the caput epididymis of the epididymis. In both species, this branch divides into two branches close to the caput epididymis and extends to the caput and corpus epididymis of the epididymis (branch numbered 3), (Figure 9).

In both species, the vessel shown with number 2 was identified as the a. epididymalis posterior, which diverged from the a. epididymalis and traveled along the cauda epididymis of the epididymis. This branch was observed to anastomose with the artery supplying the ductus deferens during its course. The branch shown with number 4 in both pictures was observed to extend between the corpus and cauda epididymis and anastomose between the epididymalis anterior and posterior (Figure 9).

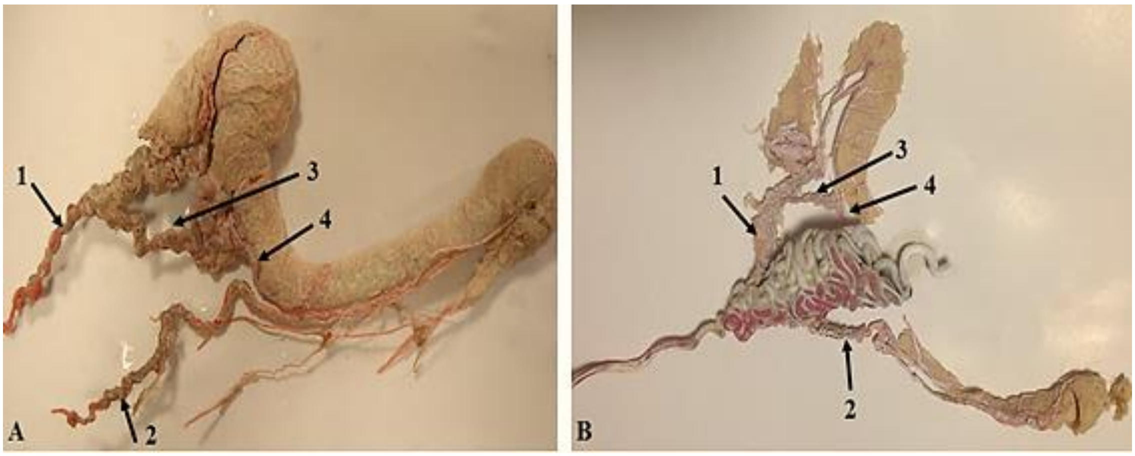

Figure 9: Distribution of the arteries supplying the epididymis (A- corrosion cast of cattle testis, B- corrosion cast of ram testis, 1- a. epididymalis anterior, 2- a. epididymalis posterior, 3- branch from a. epididymalis anterior, 4- anastomosis between a. epididymalis anterior and posterior)

Contrary to the branching seen in Figure 9, in one of the materials belonging to the ram species, it was determined that a single branch emerged from the 1/3 proximal part of the a. testicularis to the epididymis. It was determined that this branch was divided into three branches (caput epididymis, corpus epididymis, cauda epididymis) near the epididymis by showing a spiral course on the lateral surface of the artery (Figure 10).

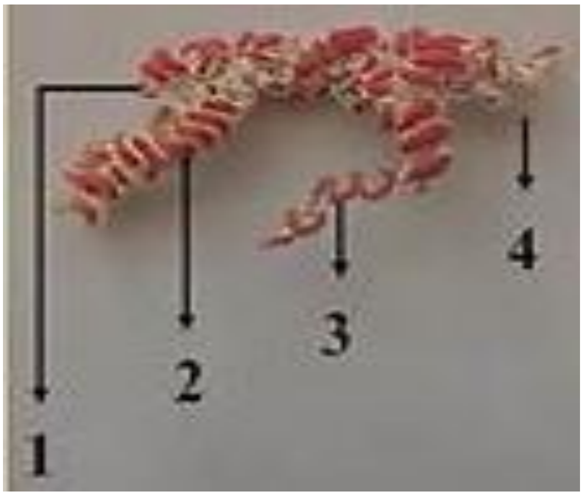

Figure 10: Course of the single branch from the a. testicularis to the epididymis of the ram's testis 1-Branch to the caput epididymis, 2- Branch to the corpus epididymis, 3- Branch to the cauda epididymis, 4-a. single branch from the testicularis to the epididymis

5- Branches of a. testicularis in the mediastinum testis

The course of the a. testicularis towards the mediastinum testis was examined in the castes of both species. In both species, the a. testicularis enters the testis by perforating the posterior part of the tunica albuginea. It was determined that it then proceeds towards the center of the testis and forms a complex vascular network in the mediastinum testis. Terminal branches (a. centripetalis and a. centrifugalis) were detected from the periphery to the center and from the center to the periphery. Dense anastomosis was observed between the branches of these vessels (Figure 11). In one of the ram testes, all of the anastomoses in the mediastinum testis were artery-arter anastomoses (Figure 11). However, we thought that one of the anastomoses in the mediastinum testis of one of the cattle testes was an artery-vein anastomosis. This anastomosis was found to course vertically, diagonally and straight from the mediastinum testis to the extremitas superior towards the venous vessels (Figure 12).

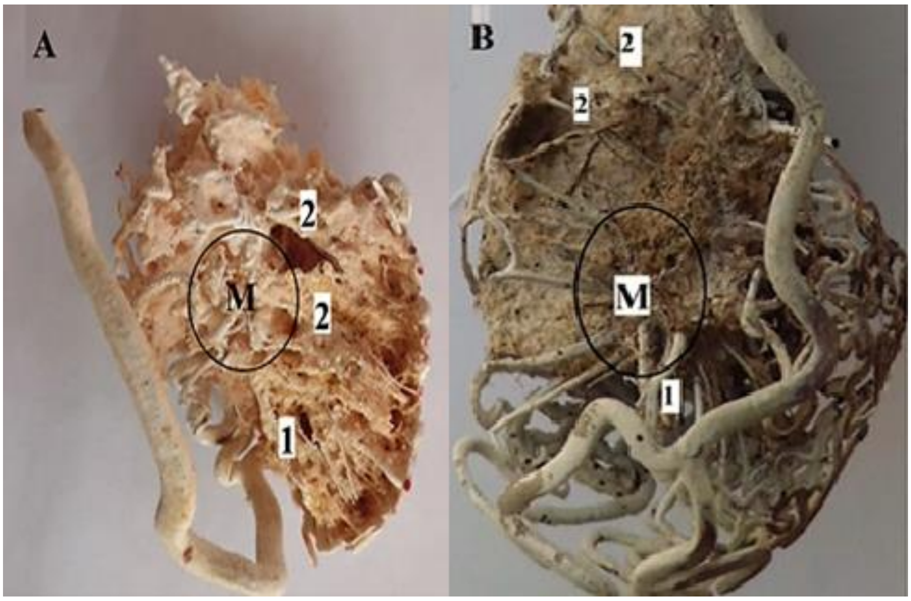

Figure 11: Location of the mediastinum testis in cattle (A) and ram (B) (M- Mediastinum testis, 1- a. centripetalis, 2- a. centrifugalis)

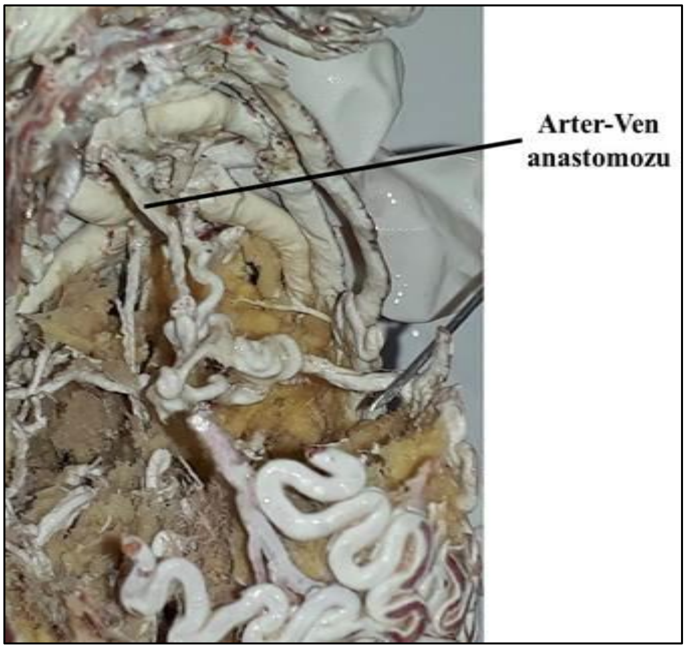

Figure 12: Artery-vein anastomosis in the mediastinum testis in cattle corrosion cast

Between the species, the branches of the a. testicularis in the mediastinum were observed to have a more prominent vascular network in cattle than in rams. In the corrosion casts of both species, the distribution of the a. testicularis superficial to the tunica albuginea, in the intratesticular space and especially in the mediastinum testis was found to have a complex structure.

6-Distribution of a. centripetalis and a. centrifugalis in the intratesticular region

In both species, the a. testicularis had terminal branches (a. centripetalis) extending from the mediastinum testis to the lobuli testis (from the periphery to the center) in the intratesticular area (Figure 13).

Figure 13: Course of A. centripetalis (A- corrosive cast of ram testis, B- corrosive cast of cattle testis)

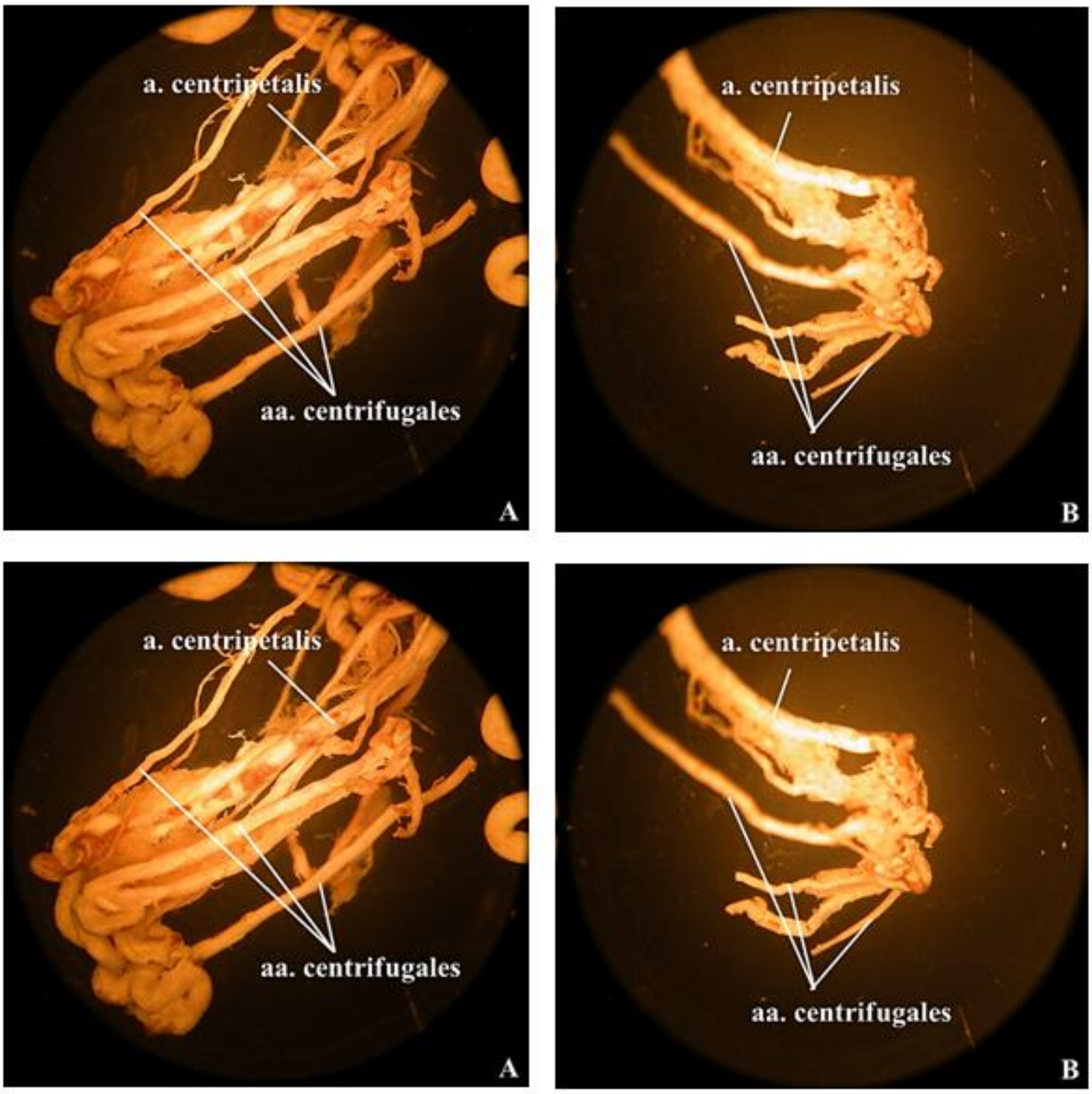

Figure 14: Course of a. centripetalis (A- corrosive cast of cattle testis, B- corrosive cast of ram testis)

The a. centripetalis of both species, which varied in number, perforated the tunica albuginea vertically and proceeded towards the parenchymal tissue towards the mediastinum testis. It was determined that a complex cluster formed in this region. A. centripetalis were seen in each cluster, originating from a a. centripetal branch and distributing radially from the center to the periphery (Figure 14).

In cattle and rams, the distribution of the a. centrigualis in the septula testis was examined microscopically because of the complexity and variable density of the vessels (Figure 15). In the analyzed images, it was seen that the a. centrifugalis divides into two or three branches while terminating towards the septula testis. Then, it was determined that there were thin branches separating from these branches. Thin capillaries were also detected from these thin branches.

It was observed that the thin capillaries formed a dense network in the septula testis. This network had a tree-like appearance in both species. Dense anastomoses were observed between the capillaries forming this tree-like vascular network. The density of branching and anastomoses was higher in cattle.

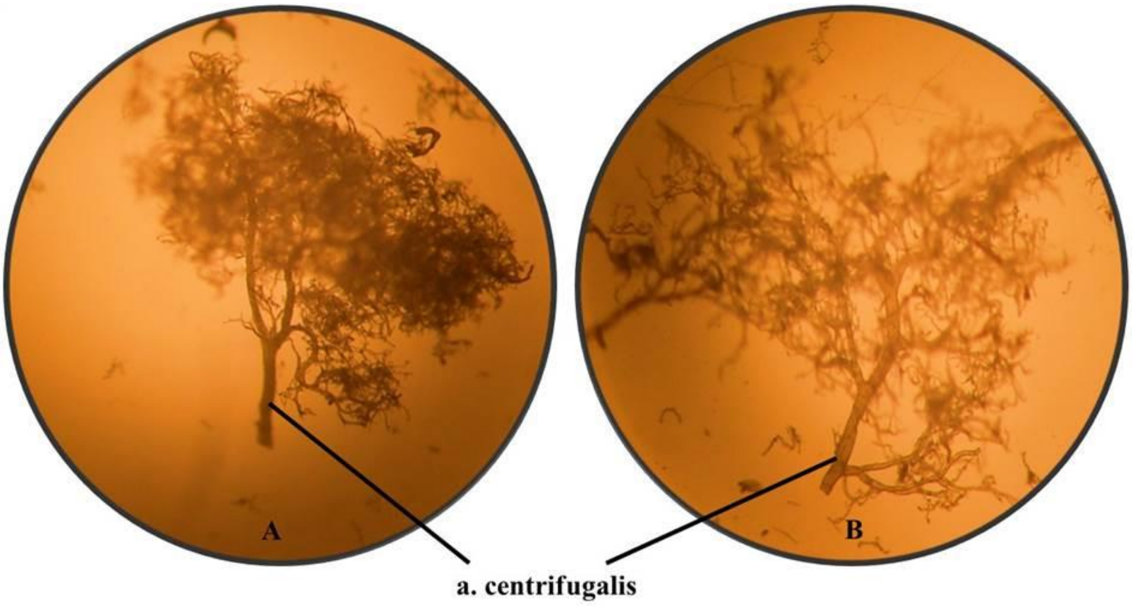

Figure 15: Distribution of a. Centrifugalis in the septula testis (A- corrosion cast of cattle testis, B- corrosion cast of ram testis)

DISCUSSION

The corrosion casting method has been used for over 500 years in many detailed studies of organ cavities and vascular structures in the body (Westberg, 1960; McLaughlin, 1961; Tompsett, 1970). The first recorded study of this method in the literature is of brain ventricles produced by Leonardo da Vinci using a molten wax. In the later period, Ruysch and Lieberkiihn used the corrosion casting method on insect larvae (Aharinejad, 1992). In addition, different materials such as low-melting metal alloys, celluloid, latex and vinyl resin have been injected into the vascular and other organ cavities to create cast models for centuries (Westberg, 1960; McLaughlin, 1961; Tompsett, 1970). In our study, a three-dimensional anatomical course of the vasculature was obtained by injecting polyester into the a. testicularis of both species.

In our study, the corrosion cast of a total of 20 a. testicularis of 10 cattle and 10 ram testes were examined. As known, a. testicularis originates from aortae abdominalis in the abdomen. After leaving the abdomen through the canalis inguinalis, it enters the testicle. In the literature, it has been reported that this vessel is straight in the course of the a. testicularis from the abdominal cavity to the funiculus spermaticus in many animals including cattle, rams, goats and humans (Hees, 1990; Carvalhal, 2000; Machado, 1996). In our study, we did not study the course of the a. testicularis from the abdominal cavity to the funiculus spermaticus.

In our study, we determined that the a. testicularis of both species showed a very tortuous distribution within the funiculus spermaticus. In some studies, it has been reported that the a. testicularis in cattle and rams forms various tortuosities during its course within the funiculus spermaticus (Polguj, 2008; Elayat *et al.*, 2016; Marco de Almeida *et al.*, 2008; Polguj, 2010; Polguj, 2011; Khalifa, 2017). Elayat *et al.*, observed that the fold density of the ram a. testicularis was more prominent compared to the cattle species (Dyce, 2010). In our study, we found that these folds were similar. We think that this difference is due to the environmental conditions and the breed of the animals.

In our literature review, we did not find any study describing the shape of the folds and curvatures of a. testicularis in the funiculus spermaticus. In our study, we determined that these folds were divided into three types. According to our study, a. testicularis funiculus spermaticus, there are three different types of folds: irregular (irregular) intertwined folds, spiral folds in the shape of the letter s and straight course folds. We have not come across a study in which the shapes of the folds are classified in this way. Therefore, we believe that this typing will be a source for future studies (Figure 4.2).

In our study, we found that the folds made by the a. testicularis in the funiculus spermaticus in the corrosion casts of both species gradually expanded from top to bottom, and as a result of this expansion, the a. testicularis took the shape of a cone. This cone was located in the extremitas superior of the testis. We found that this cone-shaped structure sat on the upper end of the testicle like a hat (Figure 4.3).

The vascular folds at the base of this cone resembled a daisy. In the studies we reviewed, it was also reported that the folds of the a. testicularis were cone-shaped (Marco de Almeida *et al.,* 2008; Polguj, 2010; Polguj, 2011; Khalifa, 2017; Dhingra, 1979; Waites, 1960; Hees, 1984). Our findings are compatible with the literature. However, Harison *et al.,* in 1949 in domestic mammals (Hees, 1984) and Rerkamnuaychoke et al. in 1991 in swine described the appearance of a. testicularis in the funiculus spermaticus as a vascular cone as we found (Rerkamnuaychoke *et al.,* 1991).

Elayat *et al.,* showed that the course of a. testicularis in the funiculus spermaticus was longer in cattle and rams than in humans (Elayat *et al.,* 2016). We did not have a study on this subject.

In our study, we found that in both species the a. testicularis extends along the margo posterior of the testis without a curve as it leaves the funiculus spermaticus, and the a. testicularis travels from the margo posterior to the extremitas inferior of the testis straight, zigzag, curved or without a specific shape (Figure 4.5).

Polguj *et al.*, In their study on 40 cattle testes, they reported that the course of the a. testicularis along the margo posterior to the cattle testes was in four different shapes: straight, curved, zigzag and mixed (Polguj, 2009). Our findings in our study are similar to those of Polguj *et al.,* We did not find any information on this subject in other sources.

It has been described in various studies that the a. testicularis shows different branching when it reaches the extremitas inferior of the testis in cattle and rams (Polguj, 2008; Marco de Almeida *et al.,* 2008; Polguj, 2009; Polguj, 2010; Khalifa, 2017).

Marco de Almeida *et al.,* determined that the a. testicularis branched into four branches at the lower end of the testis in ram testes. They found that three of these branches extend caudal to the lower end of the testis and the remaining one extends cranially. They also examined these branches in four sections as dorsimedial (DM), dorsolateral (DL), ventromedial (VM) and ventrolateral (VL) according to the regions they supply the testis (Marco de Almeida *et al.,* 2008). Polguj *et al.,* reported in 2008 and 2009 that the a. testicularis was divided into two branches at the lower end of the testis (Polguj, 2008; Polguj, 2009). In the study conducted by Khalifa *et al.,* on cattle testes in 2017, it was stated that the a. testicularis was divided into two terminal branches (Khalifa, 2017). However, in another study conducted on cattle testes by Polguj *et al.,* in 2010, it was found that there was one more accessory branch in addition to these two branches mentioned in the literature (Polguj, 2010). In our study, when we examined the course of the a. testicularis of both species, we found that this branching divided into two terminal branches near the lower end of the testis (Figure 4.7). No anastomosis was observed between the branches of the a. testicularis at the lower end of the testis. Our finding was found to be consistent with the literature.

In the articles examining the distribution of the terminal branches of A. testicularis at the lower end of the testis in the mediastinum testis, it is stated that this distribution shows differences. In some studies, it has been reported that the distribution of a. testicularis is different in humans and cattle (Jędrzejewski, 1996; Godino, 1973). These differences were reported to be due to the distribution of a. testicularis in the intratesticular area (Yalçın *et al.,* 2005). These branches are named as centrifugal and centripetal arteries in the literature (Lin, 2007; Polguj, 2008; Waites, 1960; Yalçın *et al.*, 2005). In the literature, these branches were first described by Hees *et al.*, and Hundeiker as reported by Polguj *et al.*, in 2015 and the presence of knot-like vessels in addition to these two branches has also been reported. However, they could not define the vascularization type of these vessels. Polguj *et al.*, 2015 examined intratesticular arteries in cattle. As a result of their study, they identified the branches in the same way as those described by Hees *et al.*, and Hundeiker. Polguj *et al.*, divided these branches into three groups as Type I, Type II and Type III (. Polguj *et al.*, 2015). Polguj *et al.*, in 2008 studied the corrosion casts of cattle and human testes. In a study in 2008, Polguj described the presence of centripetal arteries and centrifugal arteries extending to the mediastinum testis in cattle without spiral or knot-like anastomosis (Polguj, 2008). In our study, when we examined the distribution of a. testicularis in the intratesticular area, we observed that its distribution in the intratesticular area and especially in the mediastinum testis had a complex structure. In the corrosion casts of both species, the intratesticular branches of a. testicularis pierced the tunica albuginea perpendicularly and proceeded perpendicularly towards the mediastinum testis to the intratesticular area and formed clusters in this area. In addition, we observed that there were terminal branches extending from the mediastinum testis to the lobuli testis (from periphery to center and from center to periphery) in the intratesticular area. In our study, we found that there were dense anastomoses between the centrifugal and centripetal arteries in the intratesticular area in both species. These anastomoses had a tree-like appearance. We also found that there were artery-venous anastomoses in the mediastinum testis in addition to artery-to-artery anastomoses (Figure 4.12). Polguj *et al.*, (2008) reported that there were no anastomoses between these arteries (Polguj, 2008).

In our study, the arteries supplying the epididymis (rr. epididymales) to the cattle and ram's testis were also separated from the a. testicularis as two branches and extended towards the epididymis parallel to the funiculus spermaticus. One of these arteries supplied the caput epididymis (a. epididymalis anterior) and the other supplied the cauda epididymis (a. epididymalis posterior). In our literature review, studies on epididymis nutrition (Polguj, 2008; Polguj, 2009; Polguj, 2010; Polguj, 2011; Polguj, 2015; Yalçın *et al.*, 2005), Hees *et al.*, in 1984 in cattle, Yalcin *et al.*, in 2005 in humans, Polguj *et al.*, between 2008 and 2011 in cattle and humans, found that there were branches feeding the epididymis in the funiculus spermaticus and named them as aa. epididymales. In one of the ram testes we used in our study, we found that a single artery supplying the epididymis emerged from the proximal 1/3 of the a. testicularis. We observed that this artery divided into three branches immediately after its exit and supplied the caput epididymis, corpus epididymis and cauda epididymis (Figure 4.T0). We did not come across any literature reporting such a variation.

CONCLUSION

The corrosion caste method used in our study is still valid today and is of great importance in defining the anatomical structures of the vessels. From this point of view, it allows detailed anatomical examination of the vessels. Casts obtained with this method can be preserved for many years and do not lose their properties. Considering the difficulties in cadaver procurement in our country especially in recent years, tissue and organ samples obtained with the corrosion cast method can be used for many years in the teaching of basic medical education courses such as anatomy and histology. As a result, we found that our findings are consistent with the literature. We believe that our study will shed light on the studies to be conducted on this subject for educators, anatomists and clinicians.

Authors' Contributions

Y.T., K.A – Conceptualized, designed and wrote the draft. Y.T., K.A., E.A. - Wrote the draft. Y.T., M.M, M.O - Collected the data. Y.T., K.A, M.M, M.O: Designed the review and analysed the data; Y.T., K.A., E.A - Critically revised the draft.

Competing Interests: The authors declare no conflict of interest.

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